PB82-102294

Effect of Halogens on 'Giardia' Cyst Viability

Oregon Univ. Health Sciences Center
Portland

Prepared for

Municipal Environmental Research Lab.
Cincinnati, OH

Sep 81

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EPA-600/2-81- 174
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EFFECT OF HALOGENS ON *GIARDIA* CYST VIABILITY

by

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Grant No. R 806032

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MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
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U.S. GOVERNMENT PRINTING OFFICE: 1980-657-060/5418
TYPING GUIDE SHEET

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/2-81- 174	ORD Report	PB82 102294

4. TITLE AND SUBTITLE	5. REPORT DATE
Effect of Halogens on Giardia Cyst Viability	September 1981
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Ernest A. Meyer	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
University of Oregon Health Sciences Center Portland, Oregon 97201	BNC1A
	11. CONTRACT/GRANT NO.
	R806032
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Municipal Environmental Research Laboratory - Cin., OH Office of Research Development U.S. Environmental Protection Agency Cincinnati, Ohio 45268	Project - 8/78 - 12/80
	14. SPONSORING AGENCY CODE EPA/600/14

15. SUPPLEMENTARY NOTES

Project Officer - John C. Hoff (513) 684-7331

16. ABSTRACT

The objective of this research was to determine the effect of chlorine and other disinfecting agents on Giardia lamblia cyst viability. The agents studied included free residual chlorine and six different methods recommended for emergency disinfection of small quantities of water.

The effect of chlorine concentration on Giardia lamblia cyst viability was tested under a variety of conditions. At 25C, 1.5 mg/l chlorine for 10 min killed all cysts at pH 6, 7, and 8. At 15C, 2.5 mg/l for 10 min killed all cysts at pH 6; but at pH 7 and 8, small numbers of viable cysts remained after 30 min, but not after 60 min. At 5C, 1 mg/l chlorine for 60 min failed to kill all cysts at any pH tested. Contact with 2 mg/l chlorine for 60 min killed all cysts at pH 6 and 7, but not at pH 8. A chlorine concentration of 4 mg/l killed all the cysts at all three pH values after 60 min, but not after 30 min. A chlorine dose of 8 mg/l killed all cysts at pH 6 and 7 after 10 min contact and at pH 8 after 30 min.

The ability of six small-quantity water disinfection methods to destroy Giardia cysts was also studied. Two water qualities (cloudy and clear) and two temperatures (3° and 20 °C) were tested. At 20°C, using cloudy and clear water, all of the methods proved completely effective. But, at 3°C in cloudy water, one method (saturated iodine) was not completely effective, and in clear water, four methods (bleach, Globaline, tincture of iodine and saturated iodine) failed to destroy all the cysts. The failure of these methods appeared to be related to either an insufficient halogen residual or contact time.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Giardia Water Treatment Disinfection Chlorine Iodine Water Supply		13B

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21.
Release to Public	Unclassified	
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA Form 2220-1 (9-73)

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution; it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and provides a most vital communications link between the researcher and the user community.

This research provides the first definitive data regarding the effect of halogens on Giardia cyst viability. The method used in this study to measure cyst viability - ability to excyst - was recently developed and shown to be more reliable than previously used dye exclusion methods.

These data are of significance to those concerned with the disinfection of community water supplies as well as those concerned with small quantity water disinfection.

Francis T. Mayo, Director
Municipal Environmental Research
Laboratory

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ABSTRACT

The principal objective of this research was to determine the effect on Giardia cyst viability of the halogens chlorine and iodine, and of a number of halogen-containing agents used in the disinfection of drinking water. All of the Giardia cysts used in these studies were concentrated and purified from fecal specimens provided by humans with Giardia infections. The criterion employed for determining Giardia cyst viability was a recently developed excystation method that has been shown to be more reliable than the older dye-exclusion procedures.

The effect of chlorine concentration on Giardia lamblia cyst viability was tested under a variety of conditions. Variables employed included temperature (25°, 15°, and 5°C), pH (6, 7 and 8), and contact time (10, 30, and 60 min). Chlorine concentration ranged from 1 to 8 mg/l. Cyst survival generally increased as buffer pH increased. At 25°C, 1.5 mg/l chlorine for 10 min killed all cysts at pH 6, 7, and 8. At 15°C, 2.5 mg/l for 10 min killed all cysts at pH 6; but at pH 7 and 8, small numbers of viable cysts remained after 30 min, but not after 60 min. The following results were obtained at 5°C: 1 mg/l chlorine for 60 min failed to kill all cysts at any pH tested. Contact with 2 mg/l chlorine for 60 min killed all cysts at pH 6 and 7, but not at pH 8. A chlorine concentration of 4 mg/l killed all the cysts at all three pH values after 60 min, but not after 30 min. A chlorine dose of 8 mg/l killed all cysts at pH 6 and 7 after 10 min contact and at pH 8 after 30 min.

The ability of six small-quantity water disinfection methods to destroy Giardia cysts was also studied. Two water qualities (cloudy and clear) and two temperatures (3° and 20°C) were tested. At 20°C, using cloudy and clear water, all of the methods proved completely effective. But, at 3°C in cloudy water, one method (saturated iodine) was not completely effective, and in clear water, four methods (bleach, Globaline, tincture of iodine and saturated iodine) failed to destroy all the cysts. The failure of these methods appeared to be related to either an insufficient halogen residual or contact time.

These data demonstrate that halogen-containing agents and chlorine and iodine are capable of inactivating Giardia cysts. They also demonstrate that the effective use of these agents against Giardia requires consideration of water temperature, pH and chlorine demand.

This report was submitted in fulfillment of Contract No. R806032 by Ernest A. Meyer under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period August 1979 to September 1980, and work was completed as of September 1981.

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SECTION 1

INTRODUCTION

The problem addressed in this study relates to the control of human infections caused by parasitic protozoa in the genus Giardia. Organisms in this genus are common intestinal inhabitants of man and many other animals. In various surveys in the last 30 years, the intestinal incidence of Giardia infection in humans has ranged from 1 to 10 percent.

Humans acquire Giardia infection by ingesting the cyst form of the organism; excystation takes place in the small intestine, and the resulting trophozoites divide by binary fission and colonize the host. Trophozoites swept into the fecal stream lose their motility, round up, and are excreted as dormant, thick-walled cysts which, upon being ingested by a suitable host, are capable of excysting and establishing another intestinal infection.

Giardia infections may be silent or symptomatic. Symptoms, when they occur, usually include a fatty diarrhea (steatorrhea) with epigastric pain and gas. Giardiasis may persist for years. The organisms apparently rarely invade the host's tissues. Children, dysgammaglobulinemic individuals, and those with other immune deficits are particularly susceptible to symptomatic Giardia infections.

The epidemiology of giardiasis is similar to a number of other intestinal pathogens of man (viral, bacterial, and protozoal) in that the ultimate source of the organisms is cyst-containing fecal material, and the portal of entry is the mouth. Drinking water containing Giardia cysts is believed to have been the vehicle for a number of Giardia outbreaks in recent years in the United States and abroad, in municipal as well as in semipublic water systems. Cysts were demonstrated in the raw water of Rome, New York (1974), Camas, Washington (1976), and Berlin, New Hampshire (1977) after apparent waterborne Giardia outbreaks in these three communities (1). Other outbreaks of this disease believed to have been waterborne in origin have occurred in a number of other states, particularly in the west, including Colorado, California, Utah, Idaho, and Montana (1). The vehicle of a giardiasis outbreak in 1953-54 in Portland, Oregon, which involved an estimated 50,000 cases, has never been determined (2), nor has a 1975 outbreak in Sydney, Australia, involving hundreds of cases (3). Evidence suggests that the Giardia of lower animals, including beaver, may infect humans (4).

Inasmuch as public water supplies represent a potential vehicle for the spread of the cysts of these organisms, it is important that water be

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treated to assure that no viable Giardia cysts are present. Because disinfection is often the means by which microorganisms are inactivated in water supplies, it would be of value to know whether Giardia cysts are in fact inactivated by the usual methods of water treatment.

Early data on Giardia cyst disinfection are fragmentary and based on dye exclusion, a method recently shown to yield erroneous results. The recent development of a method of inducing excystation made possible a more accurate method of determining cyst viability and of testing the effectiveness of anti-Giardia agents. The studies reported here are the first to document the effect of halogens on Giardia cyst viability, using the ability to excyst as the criterion of viability.

It should be pointed out that excystation (the process being measured here) and viability are not precisely the same processes. While ability to excyst is a prerequisite for viability, the reverse may not be true. Thus it is possible to hypothesize a Giardia cyst capable of excystation but, damaged by chemicals or with energy stores depleted, incapable of further growth and division. It should be noted that in the studies here reported conditions are described which result in the inability of all Giardia cysts in the test suspension to excyst. Since, in practice, inactivation of all cysts is the goal, application of such conditions makes moot the question of the possible existence of Giardia capable of excystation but incapable of further growth and multiplication.

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SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

This study has refuted an earlier, generally held belief that the resistance of Giardia cysts is so great that their inactivation by agents such as halogens is not feasible. The results presented here demonstrate that easily attainable concentrations of chlorine (1 to 8 mg/l), when applied under appropriate conditons, are capable of preventing excystation, and thus presumably the inactivation of Giardia cysts.

The present study consisted of the determination of the effect on Giardia cyst viability of 1) chlorine and 2) six small-quantity water disinfection methods.

In the chlorine experiments, the following variables were studied: chlorine concentration, chlorine-cyst contact time, temperature, and pH. Within the range of these variables studied, the rate of Giardia cyst destruction was observed to increase with: (a) increasing chlorine concentration, (b) increasing time of chlorine contact with cysts, (c) increasing temperature, and (d) decreasing pH.

Only two of the six small-quantity water disinfection methods, when conducted precisely as recommended, proved capable of inactivating all of the Giardia cysts under all test conditions. In all cases, failure to inactivate all cysts occurred in cold (3°C) water; all six methods were effective in warmer (20°C) water. These results suggest that the failure of these methods to inactivate Giardia was not due to an inherent property of the disinfectant or the cyst, but to a failure of the method to provide a sufficient halogen residual and contact time.

Both aspects of this study underline the importance of considering water temperature when employing halogen compounds in the disinfection of drinking water.

This study also raises an important epidemiologic consideration where halogens are used to disinfect drinking water. At low water temperatures, the destruction of all Giardia cysts requires increasing either the amount of disinfecting agent or the contact time, or both. Increasing the halogen concentration may have two potential disadvantages: so much halogen may be required that the palatability of the water may be affected. It may also result in the formation of trihalomethanes, organic compounds believed to play a carcinogenic role. This situation is unfortunate, since low water temperatures are particularly likely to prevail where waterborne epidemics are most likely to occur, and they permit the longest

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survival of Giardia cysts -- 2 months or more.

The inactivation of Giardia cysts in water by application of halogens can thus be seen to present special problems. Where community water supplies at low temperatures are involved, it may be necessary to consider means of achieving long halogen contact times or alternate methods of water purification. Where the disinfection of small quantities of drinking water is involved, either of the two methods found effective can be employed. As an alternative, boiling the water, where means to do this are available, is an effective way of destroying these protozoan cysts (5).

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SECTION 3

MATERIALS AND METHODS

CYST SOURCES

The Giardia cysts used in these studies were obtained from several human fecal specimens, while those in the studies of small-quantity water disinfection methods were obtained from a single human fecal specimen.

The availability of a regular supply of viable G. lamblia cysts proved to be a problem in the conduct of this study. The ideal source of such cysts would be a cooperative adult with asymptomatic infection. Such a subject was not available. Most Giardia infections which are diagnosed are symptomatic and are treated promptly once diagnosis is made. While colleagues in the Clinical Pathology Department at the University of Oregon Health Sciences Center and in other health laboratories in Oregon provided us with a number of Giardia cyst-containing fecal specimens from symptomatic individuals who had acquired their infections either in Oregon or in other parts of the world, an asymptomatic Giardia cyst passer was not identified.

Not infrequently patients were identified who excreted large numbers of Giardia cysts that were incapable of excystation, even after several days of storage.

In an attempt to infect dogs, Giardia-free dogs were fed large numbers of viable G. lamblia cysts, but they failed to become infected.

Further attempts to identify Giardia-infected monkeys at the Oregon Regional Primate Research Center proved unsuccessful.

We also attempted to obtain Giardia cysts from the feces of live and freshly killed beavers to use in these experiments. With the aid of Joe Colver, a local trapper, we obtained and dissected six recently trapped beavers. We also examined fecal specimens from three live beavers, which, if infected, would have been maintained in our animal care facility. All nine animals proved negative for Giardia.

Other colleagues interested in studying Giardia report similar difficulty in establishing a consistent source of viable cysts of the Giardia duodenalis (that is, G. lamblia) type.

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CYST PURIFICATION AND STORAGE

Cysts were purified by a modification of the procedures of Roberts-Thomson et al. (6) and Sheffield and Bjorvatn (7) as follows: Feces were suspended to a thin consistency in tap water and filtered successively through 1000, 710, 500, 250 and 177 µm-aperture nylon meshes. Three to five milliliters of filtrate was placed on 3 ml of chilled 0.85 M sucrose in a 10-ml conical centrifuge tube. The tube was then centrifuged at 600 g for 5 min at room temperature in a swinging bucket rotor. The water-sucrose interface was removed, diluted 1:10 with water, and re-centrifuged for 5 min. The pellet was resuspended in 3 ml of water, and the sucrose gradient centrifugation was repeated as many times as needed to achieve the desired purity. Following this procedure, the water-sucrose interface was again removed, diluted 1:10 with water, filtered under vacuum through a 20 µm-aperture nylon mesh, and centrifuged for 5 min. This pellet was resuspended and diluted in tap water to a concentration of approximately 50,000 cysts/ml. Cyst numbers were determined by counts with a hemacytometer. In all experiments except those in which the effect of storage temperature was studied, cyst suspensions were stored at 3°C. The purification procedure could be accomplished in an hour, yielding approximately a 30-percent cyst recovery, with purity acceptable for light microscopic examination and enumeration, and apparently undiminished cyst viability.

GROWTH MEDIUM

Giardia growth medium HSP-3 derived from Meyer's HSP-1 and HSP-2 media (8) was used in the excystation and cultivation of trophozoites. This medium has the following formulation: 85 ml Hanks-phytone broth; 20 ml Seitz filter-sterilized, heat-inactivated human serum; 7.5 ml NCTC-135 (Gibco); and 1.5 ml 1.0 M $NaHCO_3$. The final pH was 7.0. For culturing trophozoites, the following antibiotics were added to HSP-3: 250,000 units potassium penicillin G, 0.02 g streptomycin sulfate, and 0.01 g gentamicin sulfate.

EXCYSTATION PROCEDURE

Unless stated otherwise, the following excystation procedure was used in all excystation experiments: One volume of purified cyst preparation (usually 0.1 ml) was added to at least 10 volumes of excystation solution, and the mixture was incubated at 37°C for 1 hour. Following incubation, the suspension was centrifuged at 600 g for 5 min at room temperature, and the pellet was resuspended in water and re-centrifuged. The pellet was then resuspended in HSP-3 (usually 0.5 ml) at 37°C. A depression slide chamber was filled with the suspension, sealed with a cover glass and paraffin-Vaseline, and incubated inverted at 37°C for 1 hour. The slide was then examined and the percentage of excystation determined. Timed experiments showed that a 1-hour incubation in HSP-3 was sufficient to allow complete escape of those trophozoites capable of excysting.

QUANTITATION OF EXCYSTATION AND STATISTICAL ANALYSIS

Excystation was quantitated by systematic examination of slide chambers at 300x on a Unitron model PH-BMIC inverted microscope. Eight fields were examined on each cover glass in the pattern shown in Figure 1.

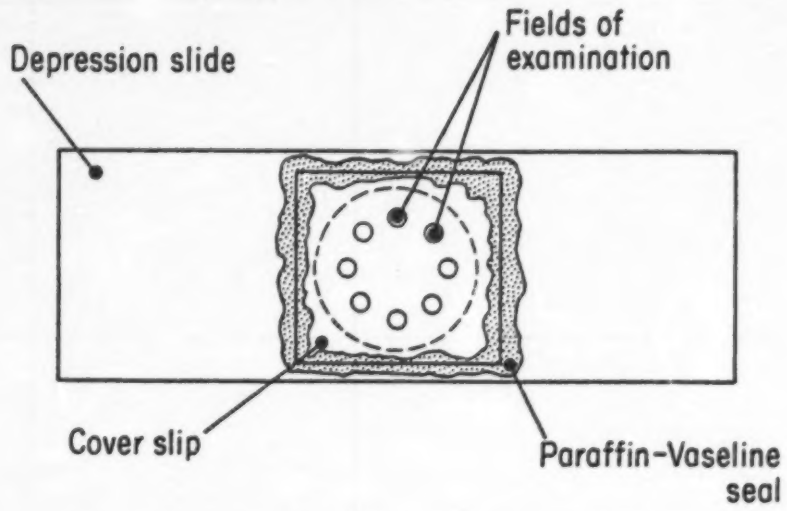

Figure 1. Pattern of the eight fields examined to determine percentage of excystation.

The fields were chosen such that they were mid-way between the outer edge and the center of the circular chamber, thus avoiding the sparsely populated outer edge and the densely populated center. This pattern was considered to be representative of the entire cover glass.

The percentage of excystation was determined by counting the number of intact cysts (IC), partially excysted trophozoited (PET), and totally excysted trophozoites (TET), and by applying the following formula:

$$\text{Percent Excystation} = \frac{TET/2 + PET}{TET/2 + PET + IC} \times 100$$

In this formula, the number of totally excysted trophozoites was divided by 2 because every cyst in which complete excystation occurred promptly yielded a pair of trophozoites. Totally excysting trophozoites rather than empty cysts were counted because: 1) empty cysts were sometimes difficult to detect because of their lack of refractility, 2) excysted trophozoites were easy to detect because of their active flagella, yet they seldom traveled appreciably, and attached to the glass almost immediately, and 3) the number of empty cysts present in a preparation as a result of causes other than excystation (e.g. death and trophozoite

disintegration) were sometimes appreciable, particularly as the preparation aged. Trophozoite multiplication did not introduce additional counting errors, since the generation time is approximately 18 hours (in M-5, a Giardia growth medium similar to HSP-3; (9)). Statistical significance was determined by Student's t-test.

EXPERIMENTS INVOLVING CHLORINE

Sørensen's sodium phosphate buffer, consisting of 0.2 M monobasic and 0.2 M dibasic sodium phosphate, was made chlorine demand-free by adding 100 µl of 5% sodium hypochlorite solution (NaOCl) to 1,000 ml of the buffer solutions followed by the exposure of these buffers to 24 h of ultraviolet irradiation to decompose the excess chlorine (10). The buffer was mixed in proportions that yielded solutions of pH 6, 7, and 8, and it was diluted with demand-free water to a 0.01 M concentration that held the pH constant and was not detrimental to the cysts.

Chlorine stock solutions were made by adding sodium hypochlorite to the diluted demand-free buffer. Chlorine residuals were determined by titration using a Wallace and Tiernan amperometric titrator (11).

Two hundred and fifty milliliters of 0.01 M demand-free chlorinated buffer was added to each of three 800-ml glass beakers with covers, maintained at either 25°, 15° or 5°C in a constant-temperature water bath, and mixed with a paddle stirrer. A cyst suspension was added to each beaker to a final concentration of approximately 650 cysts/ml. The chlorine demand of each cyst preparation was predetermined and ranged from as low as zero to 0.1 mg/l. When the cyst suspension was reduced in volume and purified as much as possible, and the chlorine demand remained above zero, additional chlorine was added to compensate for the cyst demand.

After a contact time of 10, 30, or 60 min, a 20-ml sample of each solution was removed and diluted to 200 ml with demand-free water, and the chlorine residual was verified. Simultaneously, 47 ml of each solution was removed and added to 3 ml of 0.6% solution of sodium thiosulfate to stop the action of the chlorine. The latter mixture was centrifuged at 600 x g for 5 min, and the resulting cyst pellet was exposed to an excystation procedure (12). Controls were cysts suspended in 0.01 M Sørensen's phosphate buffer without chlorine but otherwise treated the same as the chlorine solutions. The experimental protocols involved simultaneously conducting buffer and thiosulfate controls at each temperature, pH, and contact time as the chlorine treatments. Data from the buffer and thiosulfate controls, which did not affect cyst viability, are not included in this report.

Control excystation ranged from 35% to 65%, depending on the day the experiments were conducted. Based on this range of excystation, detection of $\geq$ 0.2% cyst survival was the lower limit of sensitivity for this counting procedure.

Disinfection experiments were performed in duplicate (triplicate where the quantity of cysts allowed) by counting at least 1,000 cysts at each

trial, and the percentage of excystation was determined (13). These results were expressed as percent cyst survival and were calculated as follows: Control excystation (cysts in buffer without chlorine), determined at the same time as excystation in chlorinated buffers, was arbitrarily designated as 100%, and the percent of excystation after exposure to chlorinated buffer was expressed relative to the control.

EXPERIMENTS INVOLVING SIX SMALL-QUANTITY WATER DISINFECTION METHODS

The disinfectants evaluated and their recommended applications are listed in Table 1. The quantities and the contact times (which included the time recommended for those in tablet form to dissolve) used for each disinfectant were those recommended by the manufacturer for the disinfection of drinking water or in the article describing the method.

Disinfectants were tested in cloudy and clear water at 3° and 20°C, since directions for some of these disinfectants vary depending on water clarity and temperature. These two water qualities and temperatures were chosen arbitrarily to reflect the manufacturer's directions for the use of these disinfectants under varying conditions. Cloudy water was obtained from the Willamette River at Portland; it had a pH of 7.2, a most probable number (MPN) (18) of coliform bacteria of 23/100 ml, and no detectable levels of chlorine or iodine. Sixty liters of river water was collected at the beginning of the study, stored in Nalgene containers at 3°C, and mixed before the beginning of each experiment. Water designated as clear was obtained from a water conditioning system (Continental Water Conditioning Corporation, El Paso, TX) in our laboratory. This sytem delivered pH 7.1 deionized water, which was determined to be halogen-demand free. Amperometric titration (11), employing a Wallace and Tierman amperometric titrator, was used to determine halogen residuals.

Each of six 1-liter glass beakers was filled with 1,000 ml of the water to be tested, which was allowed to equilibrate to and then maintained at 3° or 20°C. The recommended quantity of each disinfectant then was added to its respective beaker and mixed on a paddle stirrer for 15 min, by which time the tablets had dissolved. Initial halogen levels were determined, and the cyst suspension was added to each beaker to a final concentration of approximately 1,000 cysts/ml. At the end of the required contact time (Table 1), a sample of each solution was removed and the halogen residual and pH were determined. Simultaneously, 47 ml of each solution was added to 3 ml of 0.6% sodium thiosulfate solution to stop the action of the halogen. These solutions were centrifuged at 600 x g for 5 min, and the resulting cyst pellet was exposed to the excystation procedure. Controls were cysts suspended in cloudy or clear water without disinfectants, and treated otherwise the same as the disinfectant solutions; control excystation ranged from 25% to 48%, depending on the day that the experiments were conducted. The experimental protocol involved simultaneously conducting all six disinfectant treatments and the appropriate control at each water temperature and quality. Data from controls containing sodium thiosulfate, which did not affect cyst viability, are not included in this report.

TABLE 1. EMERGENCY WATER DISINFECTION METHODS EVALUATED USING THE RECOMMENDED PROCEDURES*

Disinfectant		Cloudy water (quantity/min)		Clear water (quantity/min)		Source of reference
		3°C	20°C	3°C	20°C	
Halazone	$C_7H_5Cl_2NO_4S$	5 tabs/30	5 tabs/30	5 tabs/30	5 tabs/30	14
Bleach	NaOCl (5.25%)	0.2 ml/30	0.2 ml/30	0.1 ml/30	0.1 ml/30	17
Globaline	$(NH_2CH_2COOH)_4HI{:}1.25I_2$	2 tabs/20	2 tabs/30	1 tab/20	1 tab/20	16
EDWGT+	$(NH_2CH_2COOH)_4HI{:}1.25I_2$	2 tabs/20	2 tabs/20	2 tabs/20	1 tab/10	--&
I_2(2% tincture)	I_2-HOI	0.5 ml/30	0.5 ml/30	0.25 ml/30	0.25 ml/30	17
I_2(saturated)#	I_2-HOI	26 ml/20	26 ml/20	26 ml/20	13 ml/15	15

* In those procedures where drops were recommended in the article, 0.05 ml was considered one drop: tab = tablet. Minutes denote recommended contact time.

\+ Emergency Drinking Water Germidical Tablet (EPA Reg. No. 34161-1-37257).

& Coghlan's Ltd., Winnipeg, Canada.

\# In all cases, the temperature of the saturated iodine stock solution was 20°C, and this was allowed to stand for 24 hours before use.

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Disinfection experiments were performed in duplicate, counting at least 1,000 cysts for every disinfectant (at each trial), and the percentage of excystation was determined. These results are expressed as percent of cyst mortality, calculated as follows: Control excystation was arbitrarily designated as 100%, and the percent excystation after exposure to disinfectant was expressed relative to these controls. These numbers, subtracted from 100.0% (19), are listed as percent cyst mortality.

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SECTION 4

RESULTS AND DISCUSSION

EFFECT OF CHLORINE ON *GIARDIA* CYST VIABILITY

At 25°C, there was no survival of *G. lamblia* cysts which had been exposed to concentrations of 1.5 mg/l chlorine or higher for 10 min at pH 6, 7, or 8.

At 15°C, no cysts survived a 10-min exposure to 2.5 mg/l chlorine in buffer at pH 6; but 2.3% of the cysts survived for 10 min, and 0.4% survived for 30 min in buffer at pH 7. In buffer at pH 8 at this chlorine concentration and temperature, cyst survivals of 1.2% and 0.4% were noted after 10 and 30 min, respectively. No cysts survived a 60-min exposure to 2.5 mg/l at pH 7 or 8.

At 5°C, cyst survival in general increased as the pH of the buffer increased (Figure 2). In buffer containing 1 mg/l chlorine, cyst survival after 10 min ranged from a low 35% at pH 6 to a high of 56% in buffer at pH 8.

Regardless of the pH of the buffer at 5°C, cyst survival decreased by approximately half after 10 and 30 min at a chlorine concentration of 2 mg/l, and it decreased by half again using a chlorine concentration of 4 mg/l. Virtually no cyst survival was detected at either 2 or 4 mg/l chlorine after 60 min at this temperature, regardless of the pH of the buffer.

At 8 mg/l chlorine, none of the *G. lamblia* cysts survived a 10-min exposure in buffer at either pH 6 or 7, but 0.4% of the cysts survived for 10 min at pH 8. After 30 min, no survival was observed in 8 mg/l chlorine, even at pH 8.

The chlorine concentrations that were shown in this study to be cysticidal for *G. lamblia* at 25° and 15°C were similar to those that were cysticidal for *E. histolytica* (20-22). Additional studies (if the cyst supply had permitted) would have been likely to show that shorter contact times and lower chlorine concentrations were cysticidal at 25°C.

The higher chlorine concentrations and longer contact times required to destroy *G. lamblia* cysts at low temperatures were expected (11). Chang and Fair (21) showed that increasing the water temperature decreased *E. histolytica* cyst survival at a given chlorine concentration, and

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decreased the cysticidal concentration of chlorine required.

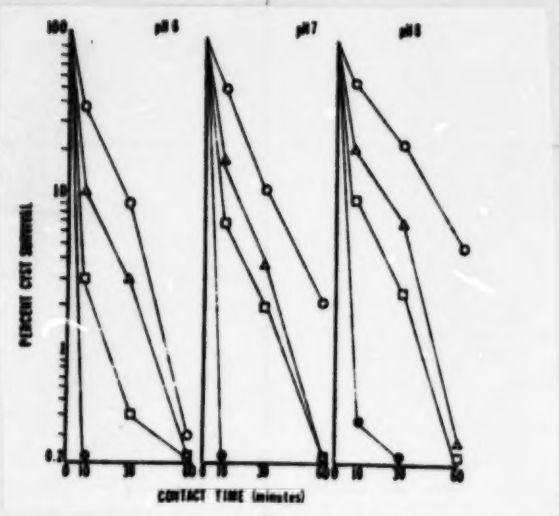

Figure 2. Representative Giardia lamblia cyst survival at 5°C in various concentrations of chlorine at 10-, 30-, and 60-minute contact times in buffer at pH 6, 7, and 8. Plotted points are means of at least two trials. Chlorine concentrations are 1 mg/l (○), 2 mg/l (△), 4 mg/l (□), and 8 mg/l (●).

Also expected was the increased cysticidal efficacy of chlorine at low rather than high pH. Chang and Fair (21) suggested that lower cysticidal concentrations of chlorine were required as the pH of the solution decreased. Stringer and Krusé (22), also using E. histolytica cysts, demonstrated that chlorine was a more effective cysticide at low rather than high pH, because at low pH, an effective cysticidal chlorine species (HOCl) predominates. A less effective chlorine species (OCl^-) predominates at high pH.

Our results clearly demonstrate that viable G. lamblia cysts can be inactivated by chlorine, and that this destruction can take place even at concentrations lower than those previously tested (11). Furthermore, the dynamics of chlorine disinfection of water containing G. lamblia cysts appear to be comparable with those of chlorine disinfection of water containing E. histolytica cysts. These studies emphasize that when chlorine is used as a cysticide against G. lamblia, attention should be given to the pH, temperature, and chlorine demand of the water being treated. The temperature consideration is especially important since: a) halogens have a reduced cysticidal activity in cold water (11,12) and b) the water temperatures in many areas where epidemic giardiasis has been reported are likely to be cold even during summer months.

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EFFECT OF SIX SMALL-QUANTITY WATER DISINFECTION METHODS ON *GIARDIA* CYST VIABILITY

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The results of representative disinfection experiments are presented in Table 2. We observed that even though liquid disinfectants were dispensed using pipettes and standardized drops, measurable variation occurred in the concentration of titratable halogen from one experiment to another. Furthermore, the titratable iodine concentration in the saturated stock solution varied from one day to the next, even though the solution was allowed to stand for 24 hours at 20°C before each use.

In clear and cloudy water at 20°C, all methods were completely effective. With cloudy water at 3°C, all of the methods except the saturated iodine inactivated >99.8% of the cysts.

In clear water at 3°C, small numbers of cysts remained viable after exposure to bleach, Globaline, 2% tincture of iodine, and saturated iodine solutions. The least effective of these were the bleach and the 2% tincture of iodine.

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The complete destruction of *Giardia* cysts in cloudy and clear water at 20°C is consistent with previous reports based on the destruction of *Entamoeba histolytica* cysts (20,21).

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The failure of Globaline and the two iodine solutions at 3°C appeared to be due to an insufficient contact time, since lower residuals of these same disinfectants inactivated *Giardia* cysts at 20°C. Whether the bleach solution failed to destroy all of the cysts because of an insufficient chlorine residual or an inadequate contact time is difficult to determine, since we tested no lower concentration at either temperature.

The recommended quantities and contact times of bleach, Globaline, and tincture of iodine solutions were determined by water clarity and not by water temperature. Though water clarity may well be related to halogen demand and thus is an important consideration, the temperature of the water to be disinfected should also be considered. The cysticidal action of halogens has been shown in this and another study (22) to decrease as the temperature decreases. Epidemiologically, adequate treatment of cold drinking water is important because *Giardia* cysts have been shown to remain viable for 2 months or longer in cold (8°C) water (13). Furthermore, inactivation of all *Giardia* cysts in drinking water is important, since ingestion of even small numbers of cysts is known to result in intestinal infection.

This study has employed the ability of *Giardia* cysts to excyst as the criterion of viability, and thus, infectivity. The ultimate test of viability - the ability of fed cysts to infect humans - while possible a quarter century ago (23), would be difficult if not impossible to conduct now. The closest one might come to such an experiment would be to determine the outcome (infection? disease?) of feeding treated cysts of the *G. lamblia* type (*G. duodenalis*) to susceptible animals. While an

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animal model for giardiasis is available and has been studied (6), the *Giardia* employed differ morphologically from the *G. lamblia* organisms.

TABLE 2. EFFECT OF DISINFECTANT TREATMENT ON *GIARDIA* CYSTS IN CLOUDY AND CLEAR WATER AT 3° AND 20°C

	3°C			20°C		
Treatment	% cyst mortality	Halogen residual*	final pH	% cyst mortality	Halogen residual	final pH
Cloudy water						
Control	0.0	0.00	7.2	0.0	0.00	7.2
Halazone	>99.8	9.56	7.8	>99.8	7.72	7.4
Bleach	>99.8	7.96	8.3	>99.8	6.36	7.8
Globaline	>99.8	9.88	5.8	>99.8	9.47	5.7
EDWGT	>99.8	9.95	5.8	>99.8	9.67	5.7
I_2(2% tincture)	>99.8	4.08	7.4	>99.8	2.79	7.1
I_2(saturated)	77.3	3.22	7.1	>99.8	3.01	7.0
Clear water						
Control	0.0	0.00	7.1	0.0	0.00	7.1
Halazone	>99.8	11.24	7.7	>99.8	11.14	7.5
Bleach	91.7	5.08	7.7	>99.8	6.20	7.9
Globaline	97.5	6.80	4.6	>99.8	5.44	5.1
EDWGT	>99.8	11.80	4.4	>99.8	5.44	5.0
I_2(2% tincture)	74.6	3.37	5.8	>99.8	3.22	5.8
I_2(saturated)	96.5	7.09	5.7	>99.8	3.15	6.0

* These values are total halogen residuals and are expressed in mg/1.

OTHER REPORTS BASED ON THIS RESEARCH

Additional reports based on the research conducted under this grant, include the following:

1. Bingham, A.K., E.L. Jarroll, E.A. Meyer, and S. Radulescu. *Giardia* sp.: Physical factors of excystation *in vitro* and excystation vs. eosin exclusion as determinants of viability. Exp. Parasitol. 47: 284-291, 1979.
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